GCA

Vol. 2 No. 12

December, 1936

# THE BOTANICAL REVIEW

**Interpreting Botanical Progress**

CONTENTS





Published Monthly at
Lime and Green Sts., Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at Lancaster, Pennsylvania, under the Act of March 3, 1879.

THE BOTANICAL REVIEW

*Editors and Publishers*

H. A. Gleason and E. H. Fulling



The Botanical Review is published monthly. Subscription rate for the United States is $3.00 per year; for Canada and other foreign countries $3.25. All subscriptions are payable in advance at par in Lancaster, Pa., or New York.

Manuscripts are prepared by contributing authors only upon invitation and on the suggestion or with the approval of the advisory editor in whose field the subject lies. Unsolicited manuscripts will not be considered. The editors, nevertheless, welcome suggestions of topics which might properly be reviewed.

Missing numbers can not be supplied unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to The Botanical Review, at Lime & Green Sts., Lancaster, Pa., or The New York Botanical Garden, Bronx Park, New York, N. Y.

# The Botanical Review

Interpreting Botanical Progress

Volume II
1936

Published Monthly at
Lime and Green Streets, Lancaster, Pa.

# The Botanical Review

CONTENTS VOLUME II, 1936

# THE BOTANICAL REVIEW

VOL. II | DECEMBER, 1936 | NO. 12


## CONTEMPORARY UNDERSTANDING OF EMBRYO-SAC DEVELOPMENT AMONG ANGIOSPERMS


KARL SCHNARF

*Botanisches Institut der Universität Wien*


According to a generally wide-spread and undoubtedly well-founded conception, the female gametophyte or embryo-sac among angiosperms is to be regarded as a structure which has passed through considerable evolutionary change and is now reduced to a few cells. It is considered as originally having been an independent generation, which among angiosperms has become an organ of the sporophyte.

It is generally observed that structures which have thus experienced considerable evolutionary change and reduction show also a high degree of variability. The embryo-sac of angiosperms is no exception and it is the purpose of the following discussion to set forth clearly the variability which occurs in its development and structure and to derive therefrom some general conclusions.

Nothing new is presented to the literature upon the subject by this paper. Attention may be directed here only to the system of embryo-sac types proposed by Palm (53) and Chiarugi (12) and to contributions published by various other authors. A new treatment may, however, be appropriate if it is concerned not only with a correlation of the old and most recent discoveries, but if it is devoted primarily to a critical distinction between well established conceptions and those that are doubtful.

It is fitting, toward this end, if we describe first those types of embryo-sac development in angiosperms which may be regarded as well established. In another part we may consider those cases that are doubtful and which merit further investigation. Certain claims may also be discussed there which heretofore have been regarded as thoroughly to be depended upon or on which particular doubt has not been cast.

This article was translated by the editors from the original German.

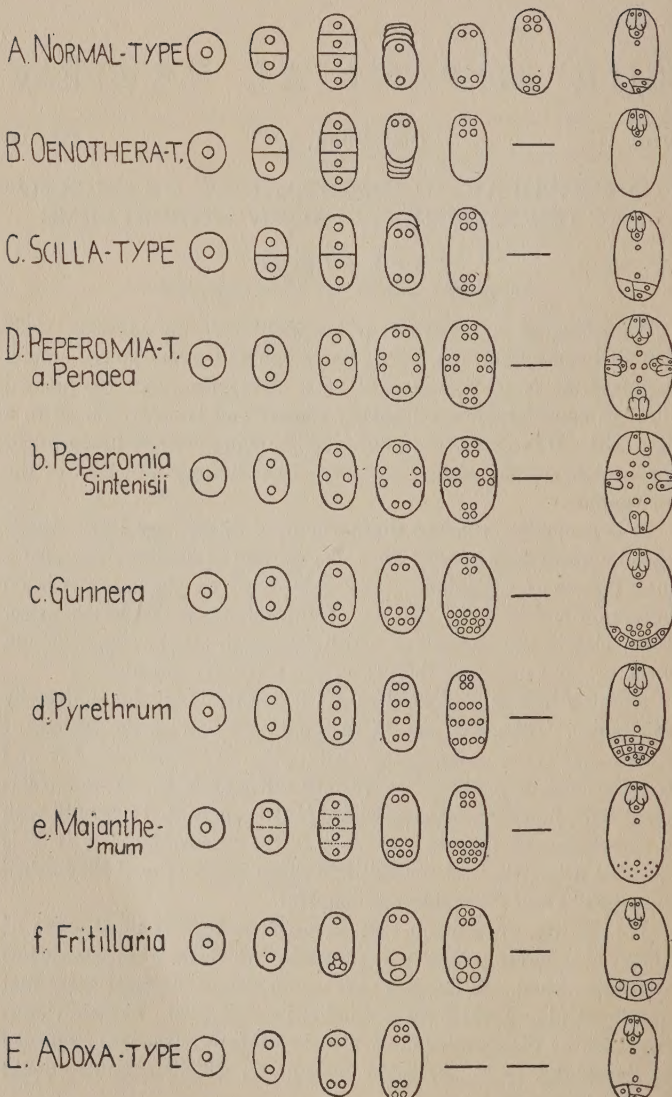


FIG. 1. Types of Embryo-sac Development among Angiosperms.

## THE WELL-ESTABLISHED TYPES OF EMBRYO-SAC

The limits and characteristics of developmental types discussed under this heading are based upon Palm's account (53). This author employs the number of successive nuclear divisions involved in the course of embryo-sac development from the embryo-sac mother-cell to formation of the egg-cell, as the principal basis for his classification. As a secondary criterion, he uses the number of macrospores involved in formation of the embryo-sac.[1]

Attention may be called at this point to the attempt of Rutgers (59) to express by a detailed system the great variation in embryo-sac development and to represent by certain formulae the types which he recognized. This attempt must be regarded as scarcely successful, primarily because the author attributes to the macrospore a new and entirely inappropriate significance. A similar attempt of Radermacher (58) can also be looked upon as unsuccessful. Chiarugi's system (12), on the contrary, contains important ideas which will be of value in the following discussion.

### THE NORMAL-TYPE

The normal-type is characterized, first by the fact that the embryo-sac mother-cell undergoes 5 divisions before formation of the egg-cell, and secondly by the fact that the embryo-sac originates from only 1 macrospore.[2] Two phases can be distinguished in this course of development (Fig. 1, A). The first leads to formation of the macrospore and consists of 2 successive divisions which involve conversion of the diploidy of the macrospore mother-cell into the haploidy of the macrospores. This phase may be briefly designated in the following discussion as spore-formation (sporogenesis of Chiarugi (12)). In the second phase, which usually is carried out by only 1 macrospore, the foundation for the fully developed and fertilizable embryo-sac is laid down by 3 successive nuclear divisions. This stage of development, the embryo-sac for-

[1] All methods of embryo-sac development described for those plants that have suffered loss of normal sexual reproduction (apomictic plants) are omitted in the following discussion. In addition to Schnarf's summary (61, *460–469*), see, in particular, Gustafson (29).

[2] I am employing the terms macrospore and macrosporangium rather than the newer and more frequently used megaspore and megasporangium because the former are older. Philological considerations do not permit rejection of old long-established terms and their substitution by new ones.

mation,[3] is characterized by considerable growth and by formation of the micropylar and the chalazal poles. Directly after the first division of the macrospore nucleus the young embryo-sac elongates considerably and a large vacuole always forms between the 2 daughter nuclei, that is, between the primary micropylar nucleus and the primary chalazal nucleus. This vacuole enlarges during the 2 subsequent nuclear divisions, after whose completion there are 4 nuclei at each pole. A complex then develops from each of these quartettes, or oangia as they are called by Chiarugi, consisting of 3 cells and 1 free nucleus. The one arising from the chalazal quartette comprises 3 antipodal cells and the lower polar nucleus while the complex at the micropylar end consists of the egg-cell, the 2 synergid cells and the upper polar nucleus. We can regard it as probable that the synergids on the one hand, and the egg-cell and the upper polar nucleus on the other hand, represent sister-nuclei; at least this is definitely shown in certain cases while no reliable observations are at hand to substantiate the idea of any other origin.

Within the normal-type we can find different variations which are exhibited in part by sporogenesis and selection of the functioning microspore and partly in embryo-sac formation.

In the first group of variations there belongs, for example, the degeneration, after the first (heterotypic) division and before the second (homotypic) division, of one of the 2 daughter-cells, the one nearer the chalazal end. In this manner a row of 3 cells is formed, only two of which, however, merit the name macrospore. It is obviously incorrect when Rutgers (59), in his description of embryo-sac development faulty also in other respects, attributes great significance to this modification in the selection of the functioning macrospore.

The normal-type shows greater variation in the second phase, that of embryo-sac formation. We find, in particular, that its antipodal half rather frequently appears altered by degeneration or advancement. The former is expressed by a slight reduction of divisions which the primary chalazal nucleus undergoes and the latter by an increase in the antipodals. It is of interest that in

[3] Within formation of the embryo-sac, Chiarugi (12) distinguished 2 stages, namely, somatogenesis, characterized by formation of the vacuole and by the first mitosis of the macrospore nucleus, and gametogenesis, during which the last 2 divisions take place.

various cases involving a greater number of antipodals in the fertilizable embryo-sacs, three of them have been shown to be of only temporary existence during the course of development.

### THE OENOTHERA-TYPE

This type, designated as the *Codiaeum*-type by Palm (53),[4] is characterized, first by the fact that the embryo-sac mother-cell undergoes 4 divisions up to formation of the egg-cell, and secondly by development of the embryo-sac from a single macrospore. This type, confined according to all indications to the Oenotheraceae, resembles the normal-type in sporogenesis; in contrast thereto, however, and with few exceptions, the micropylar nucleus is the one which always develops into the embryo-sac (Fig. 1, B). This anomalous selection of the functioning macrospore, largely fixed by heredity, is apparently associated with the complete suppression of the chalazal half of the normal embryo-sac. The functioning macrospore first undergoes considerable growth, resulting in the displacement of the macrospore-nucleus by a vacuole toward the micropylar pole of the young embryo-sac. At that point occur the 2 divisions giving rise to the normal quartette which in the fully developed embryo-sac consists of the 2 synergids, the egg-cell and 1 polar nucleus.

### THE SCILLA-TYPE

In contrast with the *Oenothera*-type, which is known in only a small number of forms, the *Scilla*-type (Fig. 1, C) has apparently developed independently from the normal type in many different classificatory groups. Its development is accomplished by 4 successive nuclear divisions from the embryo-sac mother-cell up to formation of the egg-cell. Compared with the *Oenothera*-type, however, 2 macrospores are involved in the formation of the embryo-sac. Attention may be drawn, in particular, to the fact that after the first (heterotypic) division a cross-wall is formed, but that wall formation ceases or is only transitory after the second (homotypic) division. The 4 macrospore nuclei are situated,

[4] I prefer (61, 189) to reject this name and to use in its place the expression *Oenothera*-type, primarily because of the doubtfulness of Arnold's data concerning *Codiaeum*. Lundberg (47) has shown that *Codiaeum variegatum* follows the normal type.

therefore, in 2 adjacent cells, the micropylar one of which usually degenerates.

While in the normal-type and *Oenothera*-type sporogenesis and embryo-sac formation are clearly distinct processes, in the *Scilla*-type they merge into one another indistinguishably. The second division is the last division in sporogenesis and the first one (somatogenesis according to Chiarugi (12)) of embryo-sac formation.

As in the normal-type, there are variations within the *Scilla*-type also. We may mention here only those to which attention has been called by Went's studies upon numerous members of the Podostemonaceae (72–75). Of the daughter-cells formed by the first maturation (heterotypic) division, the micropylar cell soon degenerates but its former existence is long indicated by a kind of cap over the embryo-sac. The embryo-sac is formed in the lower daughter-cell by the 2 macrospores which have arisen from the second maturation division. The 2 macrospore nuclei move toward the poles of the young embryo-sac. By 2 successive divisions the micropylar nucleus produces the 4 nuclei which form the egg apparatus and the upper polar nucleus. The primary chalazal nucleus soon degenerates, on the contrary, and formation of a quartette in the chalazal region is omitted. It is particularly noteworthy that in the Podostemonaceae the completed embryo-sac is scarcely any larger than the embryo-sac mother-cell.

Additional examples of the *Scilla*-type of embryo-sac, modified by restricted development of the antipodal region, are furnished by members of the Alismataceae investigated by Dahlgren (17), viz: *Alisma Plantago, Alisma natans, Echinodorus ranunculoides* and *Damasonium alisma.* In these cases embryo-sacs with 5 or 6 nuclei develop as a result of the fact that the primary chalazal nucleus of the embryo-sac divides only once or not at all.

### THE PEPEROMIA-TYPE

The *Peperomia*-type is characterized, first by participation of 4 macrospore nuclei in its development, and secondly by the fact that up to formation of the egg-cell the nucleus of the embryo-sac mother-cell undergoes 4 successive divisions. This type, furthermore, is composite in so far as it includes a variety of modifications. The most important of these variations which may be recognized within the type are distinguished here as its forms and

the positions of the 4 macrospores within the embryo-sac mother-cell play a significant rôle in their recognition.

1. *Penaea-Form.*—This form, observed in the Penaeaceae and certain species of *Euphorbia,* exhibits the following course of development (Fig. 1, D. a.). No walls are formed between the macrospore nuclei resulting from the 2 meiotic divisions. When this 4-nucleate cell enlarges and a central vacuole forms within it, the 4 macrospore nuclei are so distributed that one lies near the micropylar pole, another near the chalazal pole and two are laterally located opposite one another. Each one of the 4 nuclei then produces a quartette by means of 2 divisions and in the completed embryo-sac each of these quartettes consists of 3 cells and 1 free nucleus. There are, then, in addition to the egg apparatus, 4 polar nuclei and 3 entirely similar groups each of which consists of 3 cells.

2. *Peperomia-Form.* Here, too (Fig. 1, D. b.), 4 macrospore nuclei are formed in the first 2 divisions and walls develop which soon disappear, or none are formed at all. The 4 nuclei are distributed approximately as the apices of a tetrahedron and, along with simultaneous growth of the embryo-sac, they undergo 2 divisions so that altogether 16 nuclei develop which are at first free. Further development of the embryo-sac is different among various species of *Peperomia.* Formation of 2 cells, the egg-cell and the synergid, can generally be definitely established at the micropylar pole. The other 14 nuclei, on the contrary, undergo a variety of further developments. They may all remain free (*Peperomia hispidula*) or peripheral cells may be formed. In *Peperomia Sintenisii* 8 such cells develop, in other species fewer, and the number of free nuclei in these cases is correspondingly smaller.

3. *Gunnera-Form.* The form (Fig. 1, D. c.) which is characterized by species of *Gunnera* develops a 16-nucleate embryo-sac by means of 4 synchronous nuclear divisions. By means of 2 successive divisions a quartette of nuclei arises at the micropylar pole from one of the 4 macrospores, which quartette produces the egg apparatus and 1 free nucleus. Six of the other 12 nuclei give rise to 6 antipodal cells at the chalazal end, and the remaining 6 free nuclei behave as polar nuclei.

4. *Pyrethrum-Form.* Palm found the following course of development in *Pyrethrum parthenifolium.* A binucleate cell de-

velops as the result of the first meiotic division and a 4-nucleate cell from the second division (Fig. 1, D. d.). This cell elongates and the 4 macrospore nuclei lying in tandem become separated from one another by vacuoles. Each of them then undergoes 2 more divisions. From the micropylar quartette there arise the egg apparatus and the upper polar nucleus. The 12 other nuclei form the lower polar nucleus and a large antipodal apparatus consisting of one 4-nucleate cell and 7 uninucleate cells.

5. *Majanthemum-Form.* In *Majanthemum bifolium* Stenar (68) found the following course of development (Fig. 1, D. e.). Four macrospore cells first arise as a result of the 2 maturation divisions. The walls separating these cells become absorbed, however, and all 4 macrospore nuclei take part in forming the completed embryo-sac. Eight nuclei arise after the following division, two of which we find in the micropylar region, six of them in the chalazal region and a large vacuole between the two groups. After the next division the micropylar nuclei form the egg-apparatus and the upper polar nucleus. So far as the early degeneration of the antipodal region permits of determination, one of the 12 chalazal nuclei apparently becomes the lower polar nucleus and the remaining 11 form antipodal cells.

6. *Fritillaria-Form.* The description of this form (Fig. 1, D. f.) is based upon observations of Bambicioni (2) with respect to *Fritillaria persica.* In this plant the embryo-sac mother-cell becomes 4-nucleate as a result of 2 meiotic divisions without the formation of vacuoles. The 4 macrospore nuclei are then distributed approximately as has already been described for *Penaea,* but one of them soon appears at the micropylar end and the other three at the chalazal pole. All 4 nuclei then undergo division simultaneously but the 3 chalazal nuclei exhibit the special feature of fusion of their spindles. This third division results, then, in 2 haploid nuclei at the micropylar pole and 2 triploid nuclei at the chalazal pole. These 2 pairs of nuclei then become separated from one another by a large vacuole and the fourth nuclear division results in a haploid quartette (egg-apparatus and upper polar nucleus) and a lower triploid quartette (antipodals and lower polar nucleus). Since the entire development from embryo-sac mother-cell to egg-cell is consummated in 4 successive divisions and since all 4 macrospores participate in formation of the embryo-sac, I am not in-

clined to include this form in the general conception of the *Peperomia*-type. The especially remarkable and interesting feature of the *Fritillaria*-form lies in the fact that in the transition from the *Peperomia*-type an embryo-sac is produced apparently from the usual 8 nuclei.

This course of development appears to be established for a number of members of the Lilioideae (in the sense of K. Krause in Naturl. Pflanzenfam. 2. Aufl.) other than *Fritillaria persica,* e.g., *Tulipa praecox* (3), *T. Gesneriana* (4) and species of *Lilium* (3, 14). As is indicated by certain features in the older literature, the same course of development probably applies also to other members of the Lilioideae. *Erythronium dens canis* also shows the characteristic distribution of the macrospore nuclei (1 and 3) and seems to follow the *Fritillaria*-form (31). The *Fritillaria*-form is apparently generally characteristic of the Lilioideae (in K. Krause's conception of the group). This fact is not altered by the occurrence of certain exceptions as, for example, in *Tulipa Gesneriana.* In this case the distribution of nuclei previous to the third division is sometimes different, not 1 and 3, but 1 and 1 and 2, i.e., with a macrospore nucleus in the micropylar region, another approximately in the center, and two at the chalazal end; in the third division only the 2 chalazal spindles fuse, and the resulting chalazal nuclei are diploid rather than triploid. In the case of a cultivated plant, such as *Tulipa Gesneriana,* we cannot attribute particular significance to such an anomalous course of development. In the case of *Tulipa silvestris* (3) it is more difficult to pass judgment. Here the 4 macrospore nuclei gather at the micropylar pole and form, after the next division, a group of 7 cells and 1 polar nucleus.

The *Fritillaria*-form is found also in *Euphorbia dulcis*[5] (8) as well as among the Lilioideae. It is very probable, furthermore, that certain courses of development which are attributed in the of the *Peperomia*-type, e.g., *Myricaria germanica* and species of literature to the *Lilium*-type, in reality follow the *Fritillaria*-form *Piper* (62, 93, 94).

### THE ADOXA-TYPE

The *Adoxa*-type (Fig. 1, E) is to be characterized, as is the *Lilium*-type in the old sense, first by 3 successive nuclear divisions

[5] Bambicioni (2) names the development which she found in *Fritillaria* as the *Euphorbia dulcis*-type, a designation, not recognized here, which fails to account for important details of Carano's description.

from the embryo-sac mother-cell to the egg-cell, and secondly by participation of all 4 macrospore nuclei in formation of the female gametophyte. Under the heading of *Adoxa*-type, first so termed by Bambicioni, we must include all those courses of development which formerly were included in the *Lilium*-type, but only so far as they have not been shown by recent investigation to belong to the *Fritillaria*-form of the *Peperomia*-type. That there actually are forms which conform to the *Adoxa*-type, we cannot deny in view of our present understanding. *Adoxa Moschatellina* certainly belongs to this type (40, 46).

We may well refer here to a recently described modification of the *Adoxa*-type. In *Gagea lutea,* according to Stenar (67), 4 macrospore nuclei are formed in a row by the 2 maturation divisions in the embryo-sac mother-cell. Upon growth of the embryo-sac, 2 of the nuclei are displaced toward the micropylar pole and 2 toward the chalazal end. (Only occasionally is there a 1 and 3 distribution.) The third division results in 8 nuclei and these form the embryo-sac; or, as more frequently seems to be the case, the lowest macrospore nucleus degenerates and does not undergo the last division, so that only 2 antipodals appear.

## THE DOUBTFUL TYPES

### THE LILIUM-TYPE

Older authors and some of the more recent ones indicate the following type of development as occurring in numerous species, e.g., of *Lilium, Fritillaria, Tulipa, Adoxa, Piper* and of other genera. Four macrospore nuclei are formed by the 2 meiotic divisions without formation of walls; by the next division these nuclei produce an 8-nucleate embryo-sac. The *Lilium*-type is characterized, then, by 3 successive divisions and by the fact that all 4 macrospores participate in the development as well as by the fact that the two developmental phases, sporogenesis and embryo-sac formation, so sharply distinguished in the normal type, are even more completely merged than in the *Scilla*-type.

We can understand a critical attitude toward the *Lilium*-type in the sense as here given from the fact that such genera as *Lilium* and *Fritillaria,* which previous to Bambicioni's and Cooper's inves-

tigations had often been studied and were regarded therefore as unquestionable examples of the *Lilium*-type, now appear to be representatives of a modified *Peperomia*-type. Even older investigations showed certain details which did not conform entirely to the accepted development of the *Lilium*-type, such as the remarkable distribution of the 4 macrospore nuclei with one at the micropylar pole and 3 at the chalazal end. Furthermore, it was peculiar that the chalazal nuclei should frequently show a greater chromosome number than those at the micropylar end. These noteworthy facts were sometimes neglected by the older authors as anomalies, or accessory hypotheses were introduced to explain them (cf. Schnarf, 1929, pp. 205–207).

Bambicioni herself has called attention to certain findings of Frisendahl (23) in his study of *Myricaria* which indicate that the development in this plant follows that of *Fritillaria* (62, 93, 94). It may also be mentioned that certain angiosperms of entirely different relationship show similar peculiarities, which indicate that they belong not to the *Lilium*-type but to the *Fritillaria*-form of the *Peperomia*-type. As certain stages indicate, even the *Lilium*-type described for *Piper* may prove after more thorough investigation to represent the *Fritillaria*-form. No evidence is afforded us at present by the descriptions of embryo-sac development in *Adoxa moschatellina* (Jönsson, 1879; Lagerberg, 1909) or even in *Armeria* and *Statice* (Dahlgren, 1916), where the development has been recorded as of the *Lilium*-type. It would be very desirable if such claims could also be reinvestigated.[6]

Purely theoretical considerations from the viewpoint of comparative morphology also give us occasion for demanding a re-examination of all claims concerning the occurrence of the *Lilium*-type in its former sense. In nearly all courses of embryo-sac development we observe that the characteristic basic feature of the completed embryo-sac, namely, the quartette of 3 cells and 1 free nucleus, arises in such a fashion that a macrospore divides and its daughter-cells produce the quartette (normal-type), or a macrospore itself becomes the initial nucleus of the quartette (*Oenothera*-type, *Scilla*-type, *Peperomia*-type). Is it only in the old conception of the

[6] Certain researches on species of *Aloë* justify me in regarding Givelli's (25) claims as wholly unreliable when he says that *Aloë arborescens, A. Todari* var. *praecox, A. caesia, A. Varvari* and *A. ciliaris* follow the *Lilium*-type in their development.

*Lilium*-type that 2 macrospores are supposed to take part in forming the quartette, i.e., 2 entities each of which possesses the ability, phylogenetically acquired, of independently evolving a gametophyte generation?

In a paper whose object is a critical presentation of the actual facts, I cannot go so far as to characterize all claims concerning the *Lilium*-type as unfounded, and this in spite of the theoretical considerations only briefly noted here. I have already expressed this conservative viewpoint by referring to and describing the *Adoxa*-type in the first part of this paper.

### THE CYPRIPEDIUM-TYPE

This type is founded upon the description by Pace (50) of embryo-sac development as it occurs in *Cypripedium spectabile, C. parviflorum, C. pubescens* and *C. candidum.*[7] According to this description, the embryo-sac mother-cell divides into 2 cells during the first maturation division, whereas after the second division wall formation does not take place. As in the *Scilla*-type, 2 macrospore nuclei are involved in formation of the embryo-sac. A vacuole develops between them in this case also and by the following division 4 nuclei are formed, two of which are at first located at the micropylar pole and two at the chalazal pole. By a rearrangement which then ensues, one of the chalazal nuclei is supposed to move toward the 2 micropylar ones and together with them to form the egg-apparatus, while the second chalazal nucleus serves as a polar nucleus.

This description, though nicely depicted in numerous drawings, nevertheless invokes some criticism from several viewpoints. The claim, for instance, that 1 chalazal embryo-sac nucleus forsakes its position, moves toward the micropylar region and behaves there as a synergid nucleus, must be demonstrated in all its stages in order to be acceptable. Rutgers (59) has referred to this and other weaknesses of the *Cypripedium*-type in a well-founded criticism which is based primarily upon certain figures in Pace's work. Francini's (21) recently published findings respecting *Cypripedium Lecanum* indicate that this criticism is wholly justified. In Francini's species an 8-nucleate embryo-sac is developed according to

[7] *C. spectabile* = *C. reginae* = *C. hirsutum.* The last is used in Gray's Manual, ed. 7.

the *Scilla*-type, or one of fewer nuclei arises by imperfect development of the chalazal half. The mid-European *C. calceolaris* also exhibits a modification of the *Scilla*-type, as is indicated by unpublished discoveries of Ernst Oberhammer.[8] Finally, Prosina (56) found the *Scilla*-type also in *Cypripedium guttatum*.

There are still other cases reported in the literature where it is claimed that by 3 successive divisions a 4-nucleate embryo-sac arises in whose formation 2 macrospores are involved. According to Magnus (48), a 4-nucleate embryo-sac is supposed to develop from 2 macrospore nuclei in *Podostemon subulatus, Hydrobium olivaceum* and probably in *Farmeria metzgeroides*. The development in these cases is said to be such that subsequent to division one of the 2 macrospores forms the 2 synergids and the other forms the egg-nucleus and the single polar nucleus. This description appears more likely and the work of Magnus shows no stages which contradict the course of development he claims to have observed. The possibility appears to me, nevertheless, that Magnus overlooked the lower macrospore nucleus and its residuum after degeneration, structures which were found by Went in so many other species of the Podostemonaceae.

### THE DICRAEA-TYPE

The *Dicraea*-type of Palm (53) is based upon the account of *Dicraea elongata* in Mangus' work on the Podostemonaceae (48). Certain ideas raised also in connection with *Podostemon* and other plants are of interest in this connection. The *Dicraea*-type appears uncertain, however, because Magnus himself admits that insufficient material was at his disposal. Further consideration of the *Dicraea*-type consequently appears out of place.

### THE PLUMBAGELLA-TYPE

The *Plumbagella*-type, established for *Plumbagella micrantha, Plumbago capensis, P. pulchella, P. zeylanica* and *Ceratostigma plumbaginoides*, is reported to involve 2 successive divisions (15, 16). These, which at the same time are maturation divisions, supposedly give rise to 4 macrospore nuclei. One of the latter becomes the egg-nucleus, another the single antipodal cell and the other two remain free as polar nuclei. This, however, is only the general

[8] I have seen convincing preparations of this myself.

rule. Dahlgren has found a relatively large number of variations and attention may be directed here to the following: 1. No antipodals, 3 free nuclei; 2. The antipodals variously distributed and sometimes converted to a degree into synergids; 3. More than 4 nuclei; 4. Eight-nucleate embryo-sac.

Without question this variability affords a basis for criticism which would have particular significance with respect to the *Plumbagella*-type. This may be regarded in the first place as evidence that the phylogenetic development of higher plants is directed toward suppression of the gametophyte and the ultimate elimination of the antithetic alternation of generations. This interpretation has brought forth the idea that, at least in the female sex of *Plumbagella,* those evolutionary stages have been achieved which the Metazoa exhibit. Because of these facts it is fitting for those who profess these ideas that they carefully examine all arguments. It may be that in the case of *Plumbagella* we have not yet recognized the normal course of development, since we have disregarded certain observed phases as accidental variations and insignificant abnormalities.

These ideas have recently been conspicuously supported by the investigations of Haupt (20) who describes the following course of development for *Plumbago capensis,* a species which has been studied also by Dahlgren. The 4 macrospore nuclei, which arise without wall formation in the embryo-sac mother-cell, are at a certain stage so distributed that one is located at the micropylar end, another at the chalazal end and two are located at the sides. They appear to be pushed toward the wall by vacuole formation. Each of these nuclei divides into two and the resulting pairs have at first similar positions. During the course of further development one of the 2 micropylar nuclei is regularly separated by a thin membrane and the resulting cell constitutes the egg-cell. Four of the remaining 7 nuclei enlarge, move toward the center and fuse, forming the secondary embryo-sac nucleus. The remaining 3 nuclei usually degenerate but sometimes they resemble the egg-nucleus in so far as lenticular cells are formed about them. One, 2 or even 3 cells may be formed in this manner. It may be conjectured that 1 nucleus of each pair in the 8-nucleate stage behaves as a polar nucleus whereas the other becomes the nucleus of an egg-cell. Of the 4 egg-cells only the one nearest the micropyle is

constant and capable of functioning; because of premature degeneration the other three usually do not complete their development.

In any event, Haupt's findings demand a re-examination of the *Plumbagella*-type, especially so because a number of the variations described by Dahlgren fit into the course of development mentioned by Haupt.

### THE GARCINIA-TYPE

Information concerning embryo-sac development in *Garcinia Kydia* and *G. Treubi* is founded upon Treub's account (70). According to this author, the development in these species follows that of the normal-type as far as the 4-nucleate state, when 2 nuclei are located at the micropylar end and 2 others in the chalazal region. Only one of the micropylar nuclei then divides and is used in the formation of the synergids; the other nuclei are reported to remain undivided, the second micropylar nucleus functioning as the egg nucleus and the two at the chalazal end as polar nuclei. Palm (53) has already called attention to the weaknesses of this account. That only 1 micropylar nucleus should divide and that this division should never have been observed appears contrary to all other observations.

Rutgers (59) has described exactly the same course of development for *Moringa oleifera* as occurs in *Garcinia,* though his account is probably influenced by the authority of Treub. That his claims are also not well founded is apparent not only by various conspicuous shortcomings of his own work but is indicated by the more recent work of Puri (59). It has been shown, for instance, that *Moringa oleifera* follows the normal-type, and there is scarcely any doubt that a re-examination of *Garcinia* would show the same results.

### OTHER CASES

A few other accounts which in my judgment are subject to question or are founded upon insufficiently accurate data may be mentioned briefly:

1. According to W. R. Smith (66), *Clintonia borealis* follows the *Oenothera*-type. A reinvestigation is needed, since the alleged course of development does not agree with that of closely related plants.

2. *Gastrodia elata,* according to Kusano (45), follows a course of development similar to that described by Pace for *Cypripedium,*

with the difference that the embryo-sac is supposed to develop from only 1 macrospore. For a criticism see Rutgers (1923) and Schnarf (1929, p. 192).

3. *Rudbeckia laciniata.* In this species, according to Palm (53), a 4-nucleate stage without wall formation is produced by the 2 maturation divisions. One macrospore nucleus lies at the micropylar end and from it the micropylar quartette develops by means of 2 successive divisions. Two antipodals develop from the other macrospore nuclei and the third is supposed to form the third antipodal and the lower polar nucleus by division. Confirmation of this form of the *Peperomia*-type is awaited. The author himself regards his work as preliminary.

4. The conditions in *Pandanus* (9, 10, 11) must be regarded as wholly unclarified. To attempt a criticism of the available data would lead only to unreliable and worthless conjectures.

## CONCLUSIONS

This all too brief discussion, concerned primarily with the most recent findings, indicates that the elucidation and confirmation of data are not yet concluded. The foregoing discussion can only point out certain doubts and omissions in our knowledge and attempt to distinguish in a critical way the established facts from the errors and the doubtful claims. Under such circumstances general conclusions based upon available data can be made only with considerable reservation. Nevertheless, I believe, as stated in my two books on the embryology of angiosperms, that one conclusion may be regarded as fully established concerning the comparative study of embryo-sac development, and this, as has already been indicated, in spite of the by no means unimportant differences in various types of development of the embryo-sac; namely, that the uniformity in development and form of the female gametophyte is so great that it may be regarded as the most important proof of the monophyletic derivation of the angiosperms. The common features among them are, first the development according to the normal-type, and secondly, formation of the embryo-sac from quartettes (Oangia according to Chiarugi).[9]

[9] The only important attempt to explain the quartettes phylogenetically is by Porsch (55). His hypothesis that the quartette represents a transformed archegonium is, according to my opinion, in complete harmony with the entire picture which the ontogeny of the female gametophyte among angiosperms presents.

The normal-type may undoubtedly be regarded as the original for the following reasons: of all types it involves the largest number of cell divisions; spore formation and embryo-sac development are separated within it; it is of general occurrence among angiosperms, failing in no group; and finally because from it derivation of all other types is reasonable, whereas it is impossible to regard any other type as the original one from which the normal-type may have been derived.

The derived nature of the abnormal types is evidenced primarily by the fact that they occur for the most part in a great variety of plants. Shortening of the process from the normal- to the *Scilla*-type has appeared in various groups of the Liliaceae, in the Podostemonaceae, in one representative of the Rhamnaceae (*Zizyphus*), in the Boraginaceae (*Anchusa, Lycopsis*), in the Compositae (*Erigeron* spp.), among the Alismataceae and the Orchidaceae, in other words, in a great variety of groups among the angiosperms. The same is true also for different forms of the *Peperomia*-type. Phenomena of such distribution can not possibly be regarded as primitive in character. We can hardly regard the *Oenothera*-type, likewise, as primitive, though it is limited to only one family. It appears thoroughly justified, therefore, to regard the normal-type as typical of the angiosperms. The abnormal types are to be looked upon as exceptions which have appeared among various groups and are to be regarded phylogenetically as further retrogressions of the female gametophyte.[10]

A second feature of the female gametophyte, intimately connected with the general concept of the angiosperms is the quartette (Oangium), i.e., a complex of 3 cells and 1 free nucleus. The embryo-sac is a structure consisting of quartettes; the quartette forms the egg apparatus and the antipodal apparatus, and can occur once, twice or four times in the embryo-sac. In the extremely small embryo-sacs of the Podostemonaceae and the Orchidaceae

[10] Shortening of embryo-sac development has occurred also in many parthenogenetic embryo-sacs and the parthenogenetic types corresponding to the normal-type, the *Scilla*-type and the *Lilium*-type have been designated, respectively, as the *Alchemella*-type, *Taraxacum*-type and the *Antennaria*-type. The resemblance between parthenogenetic and normal sexual types is, however, wholly superficial and based upon entirely different causes. Reduction in the parthenogenetic types is founded upon the more or less extensive decline of meiosis, and is a karyological and not a phylogenetic or morphological problem (cf. 29).

it is lacking as infrequently as in those of apomictic plants. This general picture of the occurrence of quartettes is not disturbed by certain exceptions (*Peperomia, Plumbagella*). Aside from the fact that any diagnostic feature of a naturally large division can be modified or suppressed in isolated cases, these exceptional cases show, by their relationship to other plants with a typical quartette, that they belong to the angiosperms.

## Literature

Including not only those works which are mentioned in the text but also others that appear very important to the author.


1. Arnoldi, W. Zur Embryologie einiger Euphorbiaceen. Trav. Mus. Bot. Acad. St. Petersb. **9**: 136–154. 1911.
2. Bambicioni, V. Ricerche sulla ecologia e sulla embriologia di *Fritillaria persica* L. Annali Bot. **18**: 7–37. 1928.
3. Bambicioni-Mezzetti, V. Nuove richerche sull'embriologia delle Gigliacee. Annali Bot. **19**: 1–18. 1931.
4. Bambicioni V. e Giombini, A. Sullo sviluppo del gametofito femminile in *Tulipa Gesneriana* L. Annali Bot. **18**: 373–386. 1930.
5. Brown, W. H. The nature of the embryosac of *Peperomia*. Bot. Gaz. **46**: 445–460. 1908.
6. Carano, E. Ricerche sull'embriogenesi delle Asteracee. Annali Bot. **13**: 251–301. 1915.
7. ———. Nuove ricerche sulla embriologia delle Asteracee. Annali Bot. **15**: 97–196. 1921.
8. ———. Ulteriori osservazioni su *Euphorbia dulcis* in rapporto col suo compartamento apomittico. Annali Bot. **17**: 50–79. 1926.
9. Campbell, D. H. The embryosac of *Pandanus*. Bull. Torrey Bot. Club. **36**. 1909.
10. ———. The embryosac of *Pandanus coronatus*. Bull. Torrey Club. **37**. 1910.
11. ———. The embryosac of Pandanus. Ann. Bot. **25**: 373–389. 1911.
12. Chiarugi, A. Il gametofito femmineo delle Angiosperme nei suoi vari tipi di costruzione e di sviluppo. Nuovo Giorn. Bot. Ital. N. S. **34**: 1–133. 1927.
13. Cooper, D. C. Development of the embryosac of *Lilium Henryi*. Proc. Nat. Acad. Sci. **20**: 163–166. 1934.
14. ———. Macrosporogenesis and development of the embryo-sac of *Lilium Henryi*. Bot. Gaz. **97**: 346–355. 1935.
15. Dahlgren, K. V. D. Der Embryosack von *Plumbagella*, ein neuer Typus unter den Angiospermen. Arkiv. Bot. **14**: Nr. 8. 1915.
16. ———. Zytologische und embryologische Studien über die Reihen Primulales und Plumbaginales. Kgl. Svenska Vetensk. Akad. Handl. **56**. No. 4. 1916.
17. ———. Die Embryologie einiger Alismataceen. Svensk. Bot. Tidskr. **22**: 1–17. 1928.
18. Ernst, A. Ergebnis neuerer Untersuchungen über den Embryosack der Angiospermen. Verh. Schweiz. Naturf. Ges. **91**, I. 230–363. 1908.
19. ———. Zur Phylogenie des Embryosackes der Angiospermen. Ber. Deut. Bot. Ges. **26a**: 419–438. 1908.
20. Fisher, G. C. Seed development in the genus *Peperomia*. Bull. Torrey Bot. Club. **41**: 137–156, 221–241. 1914.

21. Francini, E. Primi dati di una revisione critica della sviluppo del gametofito femmineo del genere *Cypripedium*. Nuovo Giorn. Bot. Ital. **37**: 277–278. 1930.
22. ————. Ricerche embriologiche e cariologiche sul genere *Cypripedium* s. *l.* Nuovo Giorn. Bot. Ital. **38**: 154–212. 1931.
23. Frisendahl, A. Cytologische und entwicklungsgeschichtliche Studien über *Myricaria germanica* Desv. Kgl. Svenska Akad. Handl. **48**. No. 7. 1912.
24. ————. Über die Entwicklung chasmogamér und kleistogamer Blüten bei der Gattung *Elatine*. Acta Horti Gothoburgensis **3**: 99–142. 1927.
25. Givelli, F. Ricerche sullo sviluppo del gametofito femmineo e dell polline nel genere *Aloë*. Lavore Istit. Bot. Palermo **1**. 1930.
26. Guignard, L. Recherches sur la structure et la division du noyau cellulaire chez les végétaux. Ann. Sci. Nat. Bot. VI. **17**: 5–59. 1884.
27. ————. Nouvelles recherches sur le noyau cellulaire. Ann. Sci. Nat. Bot. VI. **20**: 310–372. 1885.
28. ————. L'appareil sexuel et la double fécondation dans les Tulipes. Ann. Sci. Nat. Bot. VII. **11**: 365–387. 1900.
29. Gustafson, Å. Studies on the mechanism of parthenogenesis. Hereditas **21**: 1–112. 1935.
30. Haupt, A. W. Ovule and embryosac of *Plumbago capensis*. Bot. Gaz. **95**: 649–659. 1934.
31. Hruby, K. A contribution to the cytology and embryology ot *Erythronium dens canis* L. Bull. Inter. Sci. Bohême. 1–9. 1934.
32. Johansen, D. A. Studies on the comparative morphology and cytology of the Onagraceae. Diss. Stanford Univ. 1927.
33. ————. Studies on the morphology of the Onagraceae. I. The megagametophyte of *Hartmannia tetraptera*. Bull. Torrey Bot. Club. **56**: 285–298. 1929.
34. ————. Idem. III. *Taraxia ovata*. Ann. Bot. **45**: 111–124. 1929.
35. ————. Idem. IV. *Stenosiphon linifolium*. Bull. Torrey Bot. Club. **57**: 285–314. 1931.
36. ————. Idem. V. *Zanschneria latifolia,* typical of a genus characterized by irregular embryology. Ann. New York Acad. Sci. **33**: 1–28. 1931.
37. ————. Idem. VI. *Anogra pallida*. Amer. Jour. Bot. **18**: 854–863. 1931.
38. ————. Idem. VII. *Gayophytum ramosissimum*. Bull. Torrey Bot. Club. **60**: 1–8. 1932.
39. ————. Idem. VIII. *Circaea pacifica*. Amer. Jour. Bot. **21**: 500–510. 1934.
40. Jönsson, B. Om embryosäckens utveckling hos Angiospermerna. Lunds Univ. Årsskrift **16**. 1879.
41. Johnson, D. C. On the development of *Saururus cernuus* L. Bull. Torrey Bot. Club. **27**: 365–372. 1900.
42. ————. On the development of certain Piperaceae. Bot. Gaz. **34**: 321–340. 1902.
43. ————. A new type of embryosac in Peperomia. Johns Hopkins Univ. Circ. N. S. No. 3: 19–21. 1907.
44. ————. Studies in the development of the Piperaceae. II. The structure and seed development of *Peperomia hispidula*. Amer. Jour. Bot. **1**: 323–339, 357–397. 1914.
45. Kusano, S. Experimental studies on the embryonal development in an angiosperm. Jour. Coll. Agr. Univ. Tokyo **6**. 1915.

46. LAGERBERG, J. Studien über die Entwicklungsgeschichte und systematische Stellung von *Adoxa Moschatellina* L. Kgl. Svenska Vetensk-akad. Handl. **44**. 1909
47. LUNDBERG, F. Bemerkungen über die Embryosackentwicklung von *Codiaem*. Bot. Nat. 336–339. 1931.
48. MAGNUS, W. Die atypische Embryonalentwicklung der Podostemaceen. Flora **105**: 275–336. 1913.
49. MODILEWSKI, J. Der weibliche Gametophyt der Angiospermen. (Russisch mit deutscher Zusammenfassung). Ukrainian Bot. Revue. **5**: 5–40. 1929.
50. PACE, L. Fertilization in Cyperipedium. Bot. Gaz. **44**: 353–374. 1907.
50a. ———. The gametophytes of Calopogon. Bot. Gaz. **48**: 126–137. 1909.
51. PALM, B. Über die Embryosackentwicklung einiger Kompositeen (Svensk.) Bot. Tidskr. **8**: 447–453. 1914.
52. ———. De embryologia Asteris et Solidaginis. Zur Embryologie der Gattungen *Aster* und *Solidago*. Acta Horti Bergiami **5**. No. 4. 1914.
53. ———. Studien über Konstruktionstypen und Entwicklungswege des Embryosackes der Angiospermen. Diss. Stockholm. 1915.
54. ———. Ein neuer Embryosacktypus (bei *Rudbeckia hirta* L.) Bot. Nat. 423–427. 1934.
55. PORSCH, O. Versuch einer phylogenetischen Erklärung des Embryosackes und der doppelten Befruchtung der Angiospermen. Jena. 1907.
56. PROSINA, M. N. Über die von Cypripedilum-Typus abweichender Embryosackentwicklung von Cypripedilum guttatum Sw. Planta **12**: 532–544. 1931.
57. PURI, V. A note on the embryosac and embryo of *Moringa oleifera*. Proc. Indian Acad. Sci. **1**: 279–282. 1934.
58. RADERMACHER, A. Die Gametophyten von *Nipa fruticans* und *Actinophloeus Macarthurii* Beck. Mesc. sowie ein Versuch die Systematik der Angiospermen durch die haploide Generation zu ergänzen. Ann. Jard. Buitenzorg **35**: 1–54. 1924.
59. RUTGERS, F. L. Reliquiae Treubianae. III. Embryosac und embryo of *Moringa oleifera*. The female gametophyte of angiosperms. Ann. Jard. Bot. Buitenzorg **33**: 1–66. 1923.
60. SAMUELS, J. A. Études sur les développements du sac embryonnaire et sur la fécondation du *Gunnera macrophylla*. Arch. Zellforschung **8**: 52–120. 1912.
61. SCHNARF, K. Embryologie der Angiospermen, Handbuch d. Pflanzenanatomie, herausg. von K. Linsbauer. II. Abt. 2. Teil. Berlin. 1929.
62. ———. Vergleichende Embryologie der Angiospermen. Berlin. 1931.
63. SCHNIEWIND-THIES, J. Die Reduktion der Chromosomenzahl und die folgenden Kernteilungen in den Embryosackmutterzellen der Angiospermen. Jena. 1901.
64. SCHÜRHOFF, P. N. Zur Phylogenie des Angiospermenembryosackes. Ber. Deut. Bot. Ges. **37**: 160–168. 1919.
65. SHADOWSKY, A. Types de développement des sacs embryonnaires chez les angiospermes (Russian with French résumé Jour. Soc. Bot. Russ. **10**: 353–372. 1925.
66. SMITH, W. R. The tetranucleate embryosac of *Clintonia*. Bot. Gaz. **52**: 209–217. 1911.
67. STENAR, H. Über die Entwicklung des siebenkernigen Embryosackes bei *Gagea lutea* Ker. nebst. einigen Bemerkungen über die Reduk-

tionsteilung bei *Gagea minima* Ker. Svensk Bot. Tidskr, **21**: 344–360.
68. ————. Embryologische und zytologische Beobachtungen über *Majanthemum bifolium* und *Smilacina stellata*. Arkiv. für Bot. **26**A. No. 8. 1934.
69. STEPHENS, E. L. The embryosac and embryo of certain Penaeaceae. Ann. Bot. **23**: 363–378. 1908.
70. TREUB, M. Le sac embryonnaire et l'embryon dans les angiospermes. Nouvelle série de recherches. Ann. Jard. Bot. Buitenzorg **24**: 1–17. 1911.
71. TREUB, M. ET MELLINK, J. Notice sur le développement du sac embryonnaire dans quelques angiospermes. Arch. Néerl. 15: 452–457. 1880.
72. WENT, F. A. F. C. The development of the ovule, embryosac, and egg in Podostemaceae. Rec. Trav. Bot. Néerl. **5**: 1–16. 1909.
73. ————. Untersuchungen über Podostemaceen. Verh. Akad. Wetensch. Amsterdam II. Sect. **16**. No. 1. 1910.
74. ————. Idem. II. Verh. Akad. Wetensch. Amsterdam. II. Sect. **17**. No. 2. 1912.
75. ————. Idem. III. Verh. Akad. Wetensch. Amsterdam. II. Sect. **25**. No. 1. 1926.

## EXPLANATORY NOTES

Angiosperms include all flowering plants except the relatively few 600 or so species which constitute the gymnosperms. The former are characterized by the production of ovules which ultimately develop into seeds within an entirely closed and more or less hollow structure, the ovary. Each ovule begins its development as a tiny papilla of meristematic tissue somewhere on the inner wall of the ovary while the latter is still very immature and the two structures develop simultaneously. A single cell in the subepidermal layer of each such papilla is destined to undergo a very special development, all the while remaining embedded in the surrounding tissue. This particular cell is known as the *macrospore-mother-cell* and its further development is the topic of discussion in this paper. The left-hand perpendicular row of circles in Fig. 1 represents this cell in various plants. Each smaller inner circle throughout the figure indicates a nucleus, separated in some cases from adjoining nuclei by a wall, as illustrated.

The macrospore-mother-cell divides usually into 4 macrospores, with or without walls, and in so doing its diploid $2n$ number of chromosomes is reduced to the haploid $n$ number in all subsequent nuclei up to fertilization.

One or more of the macrospores enlarge and by repeated nuclear division develop into the female gametophyte, known also as the embryo-sac, undergoing the changes described in this paper. Some of the ultimate nuclei are then situated at the *micropylar pole*, i.e., at the end of the embryo-sac nearest the micropyle or cleft between enveloping integuments of the ovule, through which cleft admission of the fertilizing pollen tube is permitted. They there constitute the so-called *egg-apparatus* which consists usually of an *egg-cell* and two additional cells known as *synergids*. Two other nuclei are located in the center of the embryo-sac and are known as the *polar nuclei* while those at the end farthest from the micropyle are the *antipodals*.

# RECENT DEVELOPMENTS IN FUNGICIDES: SPRAY MATERIALS

JOHN W. ROBERTS
*Bureau of Plant Industry, U. S. Department of Agriculture*

## INTRODUCTION

The word fungicide as used in this paper will follow the definition (91): "Any substance which may be applied to higher living plants in active growth and which will kill parasitic fungi or prevent the development of fungous diseases without killing or seriously injuring the host plant." This definition excludes applications to plants in the dormant stage and to picked fruits.

Since the principal use of fungicides is in the control of diseases of fruits such as the apple, pear, peach, plum, and grape, the principal developments in recent years have been in answer to a demand for better fungicides for these fruits.

Fungicides as here defined are used as protectants (92), that is, "They are usually effective only when applied to the plant before it has become inoculated with the spores of the pathogene." To accomplish this, it is usually necessary to apply the fungicide before the spores are present upon the plant; otherwise, some of them might germinate and infect the plant before the fungicide became effective. Thus the fungicide is a coating over the plant which is noninjurious, or nearly so, to the plant, and at the same time is toxic to spores or germ tubes of certain pathogenic fungi which may come in contact with it.

An ideal fungicide should be toxic to the pathogen, noninjurious or even beneficial to the plant sprayed, even after repeated applications, should cause no toxic accumulations in soils, should be nontoxic to men and animals, cheap and easily obtained, nonexplosive, capable of storage without deterioration, noncorrosive, easily made and applied, conveniently handled, capable of covering and sticking well, remaining active over a considerable period of time, and be insecticidal or compatible with the insecticidal sprays without lessening its effectiveness or that of the insecticide.

The common fungicides at present in use possess most of these characters to some degree, but not to the degree desired. For

instance, a spray which is sufficiently toxic to prevent infection may be unsafe to use because of the risk of severe injury to the sprayed plant. A realization of the properties which a good fungicide must have will show at once why the development of new fungicides is a hard task. When one realizes that he is applying a spray to a plant to protect it from another organism which is also a plant, he must realize that the balance between noninjuriousness to the sprayed plant and toxicity to the pathogen is very delicate indeed. Also the fact that fungicides which are sprayed upon trees in large amounts, perhaps 60 gallons per tree in one growing season, must be cheap is very discouraging to those attempting to develop new fungicides.

In recent years much work has been done to improve the older fungicides and develop new ones. Attempts have been made to find out how the various chemicals or combinations of chemicals act on fungous spores and on leaves, fruits, and other parts of the sprayed plant; also the effect of weathering on the composition, toxicity, and durability of the dried residues remaining after the fungicides have been applied.

Because the mechanism by which a fungicide kills spores appears identical with that by which it may injure the plant to which it is applied, it is evident that susceptibility to the working of this mechanism on the part of the fungus spore and resistance on the part of the sprayed plant are greatly to be desired. More efficient fungicides can be applied to injury-resistant species or varieties than to those susceptible to spray injury. On the other hand, the less effective fungicides will often control diseases without injury on species or varieties resistant to the diseases but susceptible to injury by the more effective fungicides, especially if the spores of the pathogen are easily killed. Resistance to spray injury and resistance to diseases often, therefore, influence the choice of a fungicide and lessen the risk (1) of failure to control diseases and (2) of serious damage from the spray itself.

The mechanism by which fungicides are largely prevented from entering the cells of the sprayed plant and consequently injuring them has not received the attention which it deserves. It is generally conceded that the entrance is prevented by the cuticle; but the cuticle, as an organ which largely prevents spray injury, has received little attention. It would be interesting to know how

readily the cuticle can be ruptured and whether or not wind, rain, hail, and the mechanical effect of spraying produce microscopically small ruptures through which sprays may gain access to and kill the underlying tissues. It would also be desirable to know the physical and chemical effects of the various fungicides and insecticides on the cuticle and epidermis, and the influence of weather, soils, fertilizers, pruning, and other cultural practices on the structure and development of these organs.

The problem of safe and effective fungicides for species and varieties injured by the fungicides now in use is a hard one to solve, and some of its most important aspects are strictly botanical.

Fungicides may be divided into three classes, according to their essential ingredients: (1) sulphur, (2) copper, and (3) those containing neither sulphur nor copper as essential ingredients.

## SULPHUR

Lime-sulphur (calcium polysulphide) solution, for a quarter of a century the standard spray for the control of apple-scab, often causes more injury to fruit and foliage than is desired. Many attempts have been made to modify this solution so as to lessen the danger of injury without materially reducing its fungicidal efficiency. Most of the suggested modifications consist in adding to the lime-sulphur solution chemicals having an acidic reaction, so that part or all of the polysulphide sulphur is precipitated as elemental sulphur (1, 3, 29, 37, 41, 49, 50, 51, 73). These modified lime-sulphurs appear not to have any particular advantage over a weaker solution of lime-sulphur or one of the finely divided elemental-sulphur sprays already obtainable, or a mixture of the two.

Self-boiled lime-sulphur, containing elemental sulphur as its active ingredient and the first successful fungicide for the control of peach brown-rot (*Sclerotinia fructicola*), has been almost wholly superseded by sprays in which very finely divided sulphur is held in suspension in water. Some of these sprays have been used successfully in the control of apple-scab (*Venturia inaequalis*) in place of the fungicidally more efficient but more injurious lime-sulphur solution (3, 5, 19, 29, 30, 37, 64, 89). These so-called wettable sulphurs are obtained or prepared in various ways, of which the following are examples: As a by-product in the manu-

facture of illuminating gas (89); by mixing or grinding very finely ground sulphur with a colloid such as casein or glue (84); by fusing the sulphur with a colloidal clay such as bentonite and grinding the product to an extreme fineness (30, 37); by precipitating sulphur from lime-sulphur solution (101); and by the interaction of sulphur dioxide and hydrogen sulphide (90).

Sulphur in dry form, to be applied as a dust, has been improved in recent years by finer grinding and the addition of wetting agents (94). It is used extensively on peaches for the control of brown-rot and scab, *Cladosporium carpophilum*.

The question as to how a chemical as insoluble as elemental sulphur acts as a killer of fungus spores has evoked many different answers. Where formerly it was assumed that such substances as sulphur dioxide or sulphuric acid were formed which act directly on the spores, recent investigations have failed to support these assumptions; but the investigators do not agree among themselves as to what the toxic substance is. Certain investigators produce evidence to show that pentathionic acid is formed as the toxic principle (22, 52, 53). This is disputed by others who point out that sulphur mixed with alkaline substances such as lime, which would neutralize pentathionic acid, remain toxic (86, 93). Some investigators hold to the view that the most important toxic substance is hydrogen sulphide, to which they maintain sulphur is reduced when in contact with spores or leaves (9, 22, 57, 58, 66). It is supposed that some substance, a chemical or an enzyme, produced by plants is capable of reducing sulphur to hydrogen sulphide. There is some evidence that this substance may be glutathione (9, 66). Some consider sulphur itself to be toxic (31, 86). It has been shown that the polysulphides of lime-sulphur solution may be directly absorbed by spores with the deposition of elemental sulphur (32). Since many of the derivatives of sulphur have been shown to be toxic to fungus spores, it is probable that under different conditions different compounds may be formed which act as effective fungicides. This view is further supported by the fact that the sulphur fungicides are effective with or without lime and under all combinations of weather.

Lime-sulphur solution is less apt to burn if applied at a time when it will dry quickly. Quick drying reduces the time in which

the original solution, gradually changing over to less injurious substances, chiefly elemental sulphur, is able to act with its full strength. The elemental-sulphur sprays and dusts do not cause the type of burning that lime-sulphur may cause when first applied, but under conditions of high temperatures and bright sunshine all types of sulphur sprays may burn.

Even when sulphur sprays do not burn they may cause leaves to turn yellow and fall or an entire plant to cease growth and take on a yellow, sickly appearance. For this reason the sulphur sprays are little used on potatoes, beans, grapes, and many other plants. Lime-sulphur sprays may decrease photosynthetic activities of apple leaves with an accompanying decrease of sugar content in the ripe fruit (23, 42, 43, 44).

## COPPER

Bordeaux mixture remains the standard and most used copper fungicide, as it has for the past half century, but by the so-called "instant" process the home-made mixture is more quickly and more easily made than formerly (83, 84, 85). In this process, granulated copper sulphate is dissolved directly in water in the spray tank and then hydrated lime is added. The mixture should be stirred vigorously throughout the process and during use. Factory-made bordeaux mixture is physically much better than formerly and contains a much higher percentage of copper. Bordeaux mixture frequently injures certain plants severely, such as peaches and apples, but even when there is no direct injury its use is sometimes undesirable during periods of drought, because, at least with certain plants, it increases the transpiration rate (97, 98, 99, 100). There is some evidence that this increase may be prevented by the addition of a small quantity of petroleum oil emulsion (97). However, for apple and pear leaves sprayed with copper fungicides, slower wilting and a lessened transpiration rate have been reported (69). A bordeaux mixture in which magnesium lime was used has given better results on potatoes (10), and has increased transpiration somewhat less than bordeaux mixtures in which high calcium lime was used (98). Varieties of apples and pears, the leaves of which have high osmotic values, are said to be less sensitive to copper injuries than those with leaves having low osmotic values (69). Excepting bordeaux mixture, the older

copper sprays, such as burgundy mixture and ammoniacal copper carbonate, are little used, principally because of their injuriousness to foliage. A dust composed of copper sulphate monohydrate and hydrated lime is still used, especially on truck crops, and has been changed little in recent years.

Despite the shortcomings mentioned above, copper sprays have certain advantages over the sulphur fungicides, their principal rivals for popularity. Copper is generally a better killer of fungus spores (the powdery mildews are a notable exception to this) and when it does not directly injure, it usually has no unfavorable effect on the plant sprayed. Sulphur and sulphur compounds, on the other hand, frequently cause a yellowing and stunting, even if they do not cause direct burning.

Much experimental work is now in progress to develop copper sprays less injurious than bordeaux mixture and sulphur sprays without materially reducing fungicidal properties. Because bordeaux mixture has been shown to contain considerable amounts of soluble copper (11, 12, 13, 15, 39), most of these experiments are based on the assumption that the so-called "insoluble" copper compounds will not cause serious injury to the sprayed plant, but in contact with spores will become sufficiently soluble to be either toxic to spores or to prevent germination. These "insoluble" copper materials are usually mixed with colloidal clays, colloidal clays and lime, or organic substances to keep them in suspension in water and to increase their spreading or sticking properties. The following copper compounds, most of which belong to the "insoluble coppers," have been subjected to recent experimentation and, in some cases at least, are being improved by the use of more finely divided materials and better stickers and spreaders: copper silicate (33), copper ammonium silicate (4, 37, 64, 77, 88, 102), red copper oxide (37, 38, 54, 77), copper phosphate (33, 82, 102), basic copper sulphate (29, 45, 102), copper oxychloride (102), copper sulphide (27, 28, 73), black copper oxide (33), copper resinate (24), copper zeolite (4), and Raleigh's mixture, containing copper sulphate, lye and molasses (78).

Recent work has confirmed an older idea that toxicity of copper sprays depends upon soluble copper (14, 15, 21, 34), which, if not already present in the spray in a lethal concentration, may be brought into existence by the action of spores upon the insoluble

copper of the spray residues (65, 79). It has also been shown that spores may bring about their own death by the rapid absorption of copper from "insoluble" copper spray residues (34, 79, 80). Recent work has indicated that fungus spores produce malic and possibly other acids which are capable of bringing into solution toxic quantities of copper from dried bordeaux mixture residues (68).

## OTHER FUNGICIDES

Sprays other than those containing copper or lime as essential ingredients have been extensively investigated, but only a very few have proved successful. Sodium carbonate (washing soda) has been recommended for the control of American mildew (*Sphaerotheca mors-uvae*) of gooseberries (71). Potassium permanganate is sometimes used to arrest the growth of powdery mildews, but since its action is quickly over, it must be followed by a sulphur or copper spray. Aluminum salts have been tested and sometimes recommended, but they show little promise of becoming useful fungicides (56). Alum alone or with bordeaux mixture has controlled grape downy mildew (*Plasmopara viticola*) (87). Aluminum sulphate has been added to lime-sulphur solution for the purpose of precipitating the sulphur from the calcium polysulphides, and the resulting mixture has been applied as a spray, but the aluminum probably adds little or nothing to the fungicidal properties of the mixture (1, 29, 41, 51).

Calcium is an important ingredient of the principal fungicidal sprays, lime-sulphur (calcium polysulphide) and bordeaux mixture, although it is only weakly fungicidal. It is, however, very useful in the form of milk of lime (calcium hydroxide), chiefly in the prevention of injury to sprayed plants. The lime, until it becomes completely carbonated, neutralizes the acidic substances formed in copper, sulphur, and arsenic spray residues, rendering them less harmful to the plant.

Barium sulphide formerly was used to some extent, but is now little used. It is inferior to calcium polysulphide as a fungicide (35).

Selenium compounds, because of their close chemical relationship with sulphur compounds, have received some attention, but are more injurious to plants than the sulphur compounds and

possess no advantages otherwise (95). Results of recent investigations on the effect of selenium-bearing soils on plants and on animals eating the plants would prevent the use of selenium compounds even if they were good fungicides (20, 72).

Ferrous sulphate has been used to a limited extent as a fungicide, but is generally unsatisfactory. It is more often used as a spray to correct a chlorotic condition caused by deficiency of available iron rather than as a fungicide. When added to lime-sulphur solution, ferrous sulphate produces a mixture containing ferrous sulphide and sulphur which has been used to some extent for the control of apple powdery mildew (8) and apple scab (1, 3, 50, 51, 73).

Many compounds of mercury are toxic to fungus spores even in dilute solutions, but in their present development they are too injurious to the sprayed plant to be useful as fungicides. Mercuric chloride diluted 1 part to 1000 parts of water is highly toxic to fungus spores, but is very injurious to vegetation and possesses no resistance to weathering. Silver compounds also are very toxic to fungus spores, but their cost would be prohibitive even if they could be shown to be desirable otherwise (67).

Zinc-lime, made by combining a solution of zinc sulphate with milk of lime, is used on peaches for the control of bacterial spot (*Bacterium pruni*) (81) and the prevention of arsenical spray injury (46, 48, 76, 81). It is not only noninjurious to peach trees but has a favorable effect on certain types of chlorosis. It is a weak fungicide, however, and cannot be depended upon to control scab and brown-rot. It is also used as a "corrective" spray for certain nonparasitic diseases of plants such as apple and pecan rosette, bronzing of tung-oil-tree leaves, mottle-leaf of citrus, and "little-leaf" of various fruit plants (18), when there is danger of burning from the use of zinc sulphate alone.

Studies of the elements and their compounds have shown that compounds of osmium, cerium, cadmium, lead, and thallium are toxic to fungi, but none of these is at present listed as a promising fungicide (67).

Of the vast list of organic materials, many of which are so useful as germicides, none can at present be considered as a practical fungicide. Soaps have some use in the control of powdery mildews, but are inferior to sulphur. Many of the dyes

are toxic to spores but do not otherwise possess the properties of fungicidal sprays (17, 70, 75).

Mineral oils have only slight fungicidal properties, and at their most effective strengths are apt to cause injury (61).

The tar oils at effective strengths are very injurious to foliage. (61).

The vegetable oils are weak fungicides (7, 61) but as a class are apparently less apt to cause injury than either the mineral oils or tar oils. They appear promising at the present time, not as fungicides but as spreaders to be added to fungicidal mixtures.

A wide range of manufactured hydrocarbons and their simpler hydroxyl derivatives and esters have been tested in a small way and in the form of emulsions for the control of hop mildew (*Sphaerotheca humuli*) (62). "Benzene, cyclohexane, dekalin, cymene, carvene, phellandrene, dipentine, turpentine, pine oil, geraniol, eucalyptus oil, and fenchone were phytocidal (i.e., injurious to the leaf in areas not invaded by the fungus) at the lowest concentrations at which they were fungicidal. α- and β-naphthol were fungicidal at concentrations of .15 and .2 percent., respectively, and, except in one experiment, were not phytocidal at concentrations under .5 percent. As similar results were obtained with commercial grades of α- and β-naphthol, these appear to merit further trial for the control of powdery mildews. The polyhydric phenols and the phenolic acids tested were fungicidal only at concentrations at which serious leaf injury was caused. Saligenin, salicylaldehyde, and vanillin were inactive at concentrations of about 1 percent., while paranitrophenol and picric acid were strongly phytocidal. Salicylanilide, applied in the form of its sodium salt. . . . was fungicidal at a concentration of .5 percent., almost fungicidal at one of .25 percent., and not injurious to the leaf at one of 1 percent. Suspensions containing 1 percent salicylanilide were not fungicidal, but were more active when soap was used as the spreader. . . . None of the esters tested proved likely to be of practical value as a fungicide." (From abstract in Rev. Appl. Mycol. **13**: 790. 1934). Also the fungicidal action of organic thiocyanates, resorcinol derivatives, and other selected organic compounds have been tested in the laboratory (96). Benzoic acid with a linseed-oil spreader has given good results as a spray for the control of downy mildew of tobacco (*Peronospora*

*tabacina*). Picric acid was less effective and injured the foliage (38).

While it is probably true that the fungicide of the future will be of organic nature, a great deal of work will need to be done before one is developed. At the present time none of the organic compounds or their derivatives shows promise of taking the place of the standard inorganic fungicides now in use.

## SPREADERS AND STICKERS

Many substances have been suggested for addition to fungicides to cause them to spread out or "crawl" over the surface of leaves and fruits or to adhere for a longer time. Most of these substances are of the "spreader" type and when added to fungicides cause the film of air in contact with the plant to be displaced and the droplet of spray to flatten out.

Theoretically, spreaders should be very desirable, but in actual practice they are frequently disappointing because they may leave too thin a film over the plant to give adequate protection against fungi and they may lessen the effectiveness of the fungicide (16, 55, 58), presumably by coating its particles, or reacting with it to form nontoxic compounds.

The principal substances under test are soap, glue, gelatine, casein, bentonite and other clays, bentonite-lime, the lighter mineral oils, fish oils, vegetable oils, waste sulphite liquor from paper mills, soluble resins, sulphates of the higher alcohols, salts of alkylated aryl compounds, and various other organic substances. (1, 2, 3, 6, 16, 25, 26, 29, 36, 40, 47, 49, 50, 55, 59, 60, 63, 73, 74, 77). The addition of spreaders to two of the most used fungicides, bordeaux mixture and lime-sulphur solution, which already have good spreading and adhesive qualities, has not materially increased their effectiveness, and may decrease it by lessening the thickness of the residue. The usefulness of spreaders and stickers is also limited by the fact that plants in an active growing condition must be sprayed frequently to protect the new growth, even if the residue from previous applications is still intact.

For adding wetting and spreading properties to the elemental sulphur sprays, casein, glue, bentonite, soap, and other substances have proved their value. They are essential ingredients of commonly used sulphur sprays. Spreaders and stickers may prove

useful in the development of sprays such as the "insoluble" coppers, which do not possess the spreading and adhering properties of bordeaux mixture (33, 54, 77, 82, 102).

## Literature Cited


1. Adams, J. F. Some recent results in spraying apples. Trans. Penin. Hort. Soc. **20**: 98–110. 1930.
2. ————. Fungicide No. 66. Del. State Board Agr. Bull. **25**: 40–45. 1935.
3. ————. Sulphur fungicides. Del. State Board Agr. Bull. **25**: 48–54. 1935.
4. ————. A new copper fungicide. Del. State Board Agr. Bull. **25**: 73–80. 1935.
5. Anderson, H. W. Results of disease control in 1932. Trans. Ill. State Hort. Soc. **56** (1932): 175–200. 1933.
6. Anonymous. Vegetable oils as spreaders for bordeaux mixture. Mysore Coffee Expt. Sta. Cir. 2. 3 pp. 1934.
7. Austin, M. D. and Martin, H. The incorporation of contact insecticides with protective fungicides. Potato field trials 1930–32. Jour. South-E. Agric. Coll., Wye, Kent., **32**: 49–58. 1933.
8. Ballard, W. S. and Volck, W. H. Apple powdery mildew and its control in the Pajaro Valley. U. S. Dept. Agr. Bull. 120. 26 pp. 1914.
9. Barker, B. P. T. Investigations on the fungicidal action of sulphur. IV. Third Progress Report. Ann. Rept. Agr. & Hort. Res. Sta. Univ. Bristol 1929: 130–148. 1930.
10. Blodgett, F. M., Mader, E. O., Burke, O. D., and McCormack, R. B. Total amount of copper applied and ratio of lime to copper in bordeaux as important factors in potato spraying. Abs. in Phytopath. **23**: 5. 1933.
11. Branas, J. and Dulac, J. Sur le mode d'action des bouillies cupriques au moment de leur emploi. Compt. Rend. Acad. Sci. **197**: 938–941. 1933.
12. ————, ————. Sur le mode d'action des bouillies cupriques. Rôle de la dessiccation. Prog. Agric. et Vitic. **100**: 642–644. 1933.
13. ————, ————. Sur le mode d'action des bouillies cupriques. Ann. Éc. Agric. Montpellier N. S. **23**: 104–114. 1934.
14. ————, ————. Le traitement du mildiou de la vigne par les bouillies cupriques. Compt. Rend. Acad. Agr. France **20**: 33–39. 1934.
15. ————, ————. Nouvelle contribution à l'étude du mode d'action des bouillies cupriques. Compt. Rend. Acad. Agr. France **20**: 500–505. 1934.
16. ————, ————. Sur quelques effets des produits ajoutés aux bouillies cupriques. Rev. Path. Veg. **22**: 13–18. 1935.
17. Boutaric, A., Doladilhe, M. and Piettre, M. Sur l'emploi des matiéres colorantes comme agents anticryptogamiques, dans les maladies des végétaux. Compt. Rend. Acad. Agri. France **18**: 819–824. 1932.
18. Brenchley, W. E. The essential nature of certain minor elements for plant nutrition. Bot. Rev. **2**: 173–196. 1936.
19. Burrell, A. B. and Parker, R. G. Field trials with lime-sulphur and Koppers flotation sulphur in apple-scab control. Proc. Amer. Soc. Hort. Sci. 1932, **29**: 98–102. 1933.
20. Byers, H. G. Selenium occurrence in certain soils in the United States, with a discussion of related topics. U. S. Dept. Agr. Tech. Bull. 482. 47 pp., 1935.

21. DELAGE, B. The solubility of copper in anticryptogamic products and and its importance. Chim. et Indus. (Special No.) **27**: 853–858. 1932. [Abstract in Chem. Abs. **26**: 3608–3609. 1932.]
22. DEL GUIDICE, E. Alcune esperienze sull 'azione anticrittogamica dello solfo. Bol. R. Staz. Pat. Veg. N. S. **11**: 128–137. 1931.
23. DELONG, W. A. and PICKETT, A. D. On a possible effect of fungicides upon the composition of apples. Science N. S. **73**: 649–650. 1931.
24. DEONG, E. R. Fungicidal values of pine-tar oil and copper resinate. Phytopath. **22**: 861–864. 1932.
25. DESRUE, A. Les mouillants en agriculture. Rev. Vitic. **78**: 405–411. 1933.
26. ————. Bouillies mouillantes et mouillants. Progr. Agr. Vitic. **104**: 228–231, 257-262. 1935.
27. DULAC, J. Utilisation des propriétés du sulfure de cuivre. Compt. Rend. Acad. Agr. France **20**: 650–652. 1934.
28. ————. Étude des conditions de la meilleure efficacité d'une bouillie anticryptogamique au sulfure de cuivre. Progr. Agr. Vitic. **103**: 345–348. 1935.
29. DUTTON, W. C. Spray injury studies. I. Injuries from summer applications on apples. Mich. Agr. Exp. Sta. Spec. Bull. **218**: 1–68. 1932.
30. ————. Control of apple scab. 62d Ann. Rept. (1932) Mich. State Hort. Soc.: 54–57. 1933.
31. FONZES-DIACON. Rôle physique et chimique des rayons ultra-violets sur le soufre sublimé. Action du soufre sur l'Oïdium. Progr. Agr. et Vitic. **95**: 155–158. 1931.
32. GOLDSWORTHY, M. C. The fungicidal action of liquid lime-sulphur. Phytopath. **18**: 355–360. 1928.
33. ———— and GREEN, E. L. Some promising fungicides. Phytopath. **23**: 561–562. 1933.
34. ————, ————. Availability of copper in bordeaux mixture residues and its absorption by conidia of Sclerotinia fructicola. Jour. Agr. Res. **52**: 517–533. 1936.
35. GOODWIN, W., MARTIN, H., AND SALMON, E. S. Polysulphide sulphur in relation to the fungicidal efficiency of certain spray materials. Ann. Appl. Biol. **17**: 127–136. 1930.
36. HAMILTON, J. M. Studies of the fungicidal action of certain dusts and sprays in the control of apple scab. Phytopath. **21**: 445–523. 1931.
37. ————. Studies on apple scab and spray materials for its control in the Hudson Valley. N. Y. State Agr. Exp. Sta. Tech. Bull. **227**: 3–56. 1935.
38. HENDERSON, R. G. Promising fungicides for tobacco downy mildew control. Abs. in Phytopath. **26**: 94. 1936.
39. HOCKENYOS, G. L. Solubility of bordeaux. Phytopath. **21**: 231–234. 1931.
40. ————, AND IRWIN, G. R. Studies on bordeaux deposition. Phytopath. **22**: 857–860. 1932.
41. HOCKEY, J. F. Cooperative investigation of fungicides for apple orchards. Rept. Dominion Botanist for 1930. Div. Bot., Canada Dept. Agri., p. 106.
42. ————, AND WARD, R. W. Studies in apple storage. I. The influence of fungicides on flavour and sugar content. Sci. Agr. **12**: 709–715. 1932.
43. HOFFMAN, M. B. The effect of certain spray materials on the carbon dioxide assimilation by McIntosh apple leaves. Proc. Amer. Soc. Hort. Sci., 1932. **29**: 389–393. 1933.
44. ————. Carbon dioxide assimilation by apple leaves as affected by lime-sulphur sprays. II. Field experiments. Proc. Amer. Soc. Hort. Sci. 1933. **30**: 169–175. 1934.

45. Holland, E. B., Dunbar, C. O., Gilligan, G. M., and Doran, W. L. The preparation and effectiveness of basic copper sulphate as a fungicide. Mass. Agr. Exp. Sta. Bull. **254**: 124–149. 1929.
46. Hurt, R. H. The prevention of arsenical injury to peach twigs and foliage in Virginia. Phytopath. **21**: 1204. 1931.
47. ————. The waste sulphite material of paper mills as an adjuvant to certain spray materials. Va. Agr. Exp. Sta. Bull. 277. 10 pp. 1931.
48. Kadow, K. J., and Anderson, H. W. The rôle of zinc sulphate in peach sprays. Ill. Agr. Exp. Sta. Bull. **414**: 207–255. 1935.
49. Kearns, H. G. H., Marsh, R. W., and Martin, H. Combined washes. Progress report. Rept. Agric. & Hort. Res. Sta. Bristol **1934**: 109–125. 1935.
50. Kelsall, A. The iron-sulphate and lime-sulphur mixture as a spray. Can. Chem. Metall. **19**: 239. 1935.
51. ————, Hockey, J. F., and Walter, G. P. Experiments with new spray mixtures. Ann. Rept. Pom. & Fruit Grow. Soc. Quebec **36**: 26–37. 1930.
52. Liming, O. N. The relation of pentathionic acid and its component constituents to the toxicity of sulphur fungicides. Phytopath. **22**: 143–165. 1932.
53. ————. The preparation and properties of pentathionic acid and its salts; its toxicity to fungi, bacteria, and insects. Phytopath. **23**: 155–174. 1933.
54. Magie, R. O., and Horsfall, J. G. Relative adherence of cuprous oxide and other copper fungicides. Abs. in Phytopath. **26**: 100–101. 1936.
55. Manns, T. F., and Adams, J. F. Department of plant pathology. Ann. Rept. Del. Agr. Exp. Sta. for 1932 (Bull. 179): 43–55. 1932.
56. Manzoni, L. Prove con l'anticrittogamico 'Italia.' Reprint from Ann. Staz. Sper. Vitic. Conegliano **3** (2): 10 pp. 1930.
57. Marsh, R. W. Investigations of the fungicidal action of sulphur. III. Studies on the toxicity of sulphuretted hydrogen and on the interaction of sulphur with fungi. Jour. Pomol. & Hort. Sci. **7**: 237–250. 1929.
58. Martin, H. The hydrolysis of sulphur in relation to its fungicidal activity. Jour. Agr. Sci. **20**: 32–44. 1930.
59. ————. The present uses and future development of spray spreaders. Hort. Educ. Assoc. Yearbook **1**: 76–84. 1932.
60. ————. Studies upon the copper fungicides. II. Some modifications of bordeaux mixture designed to overcome practical difficulties in its application. Ann. Appl. Biol. **20**: 342–363. 1933.
61. ————, and Salmon, E. S. The fungicidal properties of certain spray fluids. VIII. The fungicidal properties of mineral, tar, and vegetable oils. Jour. Agr. Sci. **21**: 638–658. 1931.
62. ————, ————. The fungicidal properties of certain spray fluids. XI. Synthetic solvents. Jour. Agr. Sci. **24**: 469–490. 1934.
63. ————, ————, and Ware, W. M. Spraying experiments against pear scab. Jour. South E. Agr. Coll., Wye, Kent **34**: 145–154. 1934.
64. Martin, W. H. Plant pathology. 53rd and 54th Annual Reports. First Bienn. Rept.) N. J. Agr. Exp. Sta.: 57–66. 1933.
65. McCallan, S. E. A. Studies on fungicides. III. The solvent action of spore excretions and other agencies on protective copper fungicides. Cornell Agr. Exp. Sta. Mem. **128**: 25–79. 1930.
66. ————, and Wilcoxon, F. The fungicidal action of sulphur. II. The production of hydrogen sulphide by sulphured leaves and spores and its toxicity to spores. Contr. Boyce Thompson Inst. **3**: 13–38. 1931.

67. ———, ———. Fungicidal action and the periodic system of the elements. Contr. Boyce Thompson Inst. **6**: 479–500. 1934.
68. ———, ———. The action of fungous spores on bordeaux mixture. Abs. in Phytopath. **26**: 101–102. 1936.
69. Menzel, K. C. Untersuchungen der schädigenden Wirkungen kupferhaltizer Spritzmittel. Angew. Bot. **17**: 225–253. 1935.
70. Meyer, A. Sur l'emploi des colorants et de diverses substances organiques dans la lutte contre les maladies cryptogamiques, en particulier le mildiou de la vigne. Rev. Vitic. **76**: 197–202. 1932.
71. Muskett, A. E., and Turner, E. The control of American gooseberry mildew in northern Ireland. Part II. Jour. Min. Agr. Northern Ireland **3**: 83–96. 1931.
72. Nelson, E. M., Hurd-Karrer, A. M., and Robinson, W. O. Selenium as an insecticide. Science n. s. **78**: 124. 1933.
73. Osterwalder, A. Schorfbekämpfungsversuche mit Schwefelkalkbrühe und verschiedenen Zusätzen. Schweiz. Zeits. für Obst.- und Weinbau **36**: 446–454. 1928.
74. Pallier, A. La résine colloïdale comme mouillant et fixatif. Rev. Vitic. **76**: 92–94. 1933.
75. Pastac, I. Constitution of organic dyestuffs and their anticryptogamic action. Chim. et Indus. (Special No.) **27**: 851–861. 1932 (French). Abs. in Chem. Abs. **26**: 3608. 1932.
76. Poole, R. F. Arsenical injury on the peach. N. C. Exp. Sta. Tech. Bull. 49: 13 pp. 1935.
77. ———. Effects of some copper compounds on the control of *Bacterium pruni* and on the peach tree. Abs. in Phytopath. **26**: 105. 1936.
78. Raleigh, W. P. A homemade colloidal copper spray. Abs. in Phytopath. **23**: 29. 1933.
79. Ribereau-Gayon, J. Sur le mécanisme de l'action des composés cupriques contre le mildiou. Compt. Rend. Acad. Agr. France **19**: 550–555. 1933.
80. ———. Sur le traitement du mildiou de la vigne par les bouillies cupriques. Compt. Rend. Acad. Agr. France **20**: 184–189. 1934.
81. Roberts, J. W., and Pierce, Leslie. Zinc-lime, a fungicide for the peach. Phytopath. **22**: 415–427. 1932.
82. Roberts, J. W., Pierce, L., Smith, M. A., Dunegan, J. C., Green, E. L., and Goldsworthy, M. C. Copper phosphate mixture: A promising fungicide. Abs. in Phytopath. **25**: 32–33. 1935.
83. Robinson, R. H. Powdered Bordeaux mixture. Indus. & Eng. Chem. **15**: 941–942. 1923.
84. ———. Sprays, their preparation and use. Ore. Agr. Exp. Sta. Bull. 259: 5–27. 1930.
85. Schneiderhan, F. J. Instant bordeaux. W. Va. Agr. Exp. Sta. Cir. 60. 8 pp. 1932.
86. Sempio, C. Meccanismo di azione dello zolfo mella lotta contro le Erisifacee. Ann. Tecnica Agraria **5**: 4–60. 1932.
87. Serveille, J. Encore l'alun. Progr. Agric. & Vitic. **98**: 525–526. 1932.
88. Sessions, A. C. Fungicide adjustment. Indus. & Eng. Chem. **28**: 287–290. 1936.
89. Smith, M. A. The control of certain fruit diseases with flotation sulphurs. Phytopath. **20**: 535–553. 1930.
90. Tisdale, L. E. Colloidal sulphur: preparation and toxicity. Ann. Mo. Bot. Gard. **12**: 381–418. 1925.
91. Waite, M. B. Fungicide as a term commonly used has three definitions. U. S. Dept. Agr. Yearbook 1927: 341–342. 1928.
92. Whetzel, H. H. and McCallan, S. E. A. Studies on fungicides. I. Concepts and terminology. Cornell Agr. Exp. Sta. Mem. 128: 3–7. 1930.

93. Wilcoxon, F. and McCallan, S. E. A. The fungicidal action of sulphur. I. The alleged rôle of pentathionic acid. Phytopath. **20**: 391–417. 1930.

94. ———, ———. The fungicidal action of sulphur. III. Physical factors affecting the efficiency of dusts. Contr. Boyce Thompson Inst. **3**: 509–528. 1931.

95. ———, ———. The fungicidale action of sulphur. IV. Comparative toxicity of sulphur, selenium, and tellurium. Contr. Boyce Thompson Inst. **4**: 415–424. 1932.

96. ———, ———. Fungicidal action of organic thiocyanates, resorcinol derivatives, and other organic compounds. Contr. Boyce Thompson Inst. **7**: 333–339. 1935.

97. Wilson, J. D. and Runnels, H. A. Some effects of bordeaux mixture on transpiration. Bi-month. Bull. Ohio Agr. Exp. Sta. 18: 147–151. 1933.

98. ———, ———. The relative influence of calcium and magnesium in bordeaux mixture on transpiration rate, II. Bi-month. Bull. Ohio Agr. Exp. Sta. 19: 175–179. 1934.

99. ———, ———. Transpirational response of various plants to bordeaux mixture, Ohio Agr. Exp. Sta. Bi-month. Bull. 19: 198–202. 1934.

100. ———, ———. The relation of time to the effect of bordeaux mixture on transpiration. Ohio Agr. Exp. Sta. Bi-month. Bull. **20**: 120–124. 1935.

101. Young, H. C. The sulphur sprays. Proc. Ohio State Hort. Soc. 1924: 85–91. 1924.

102. ———, and Beckenbach, J. R. Insoluble copper compounds as substitutes for bordeaux. Abs. in Phytopath. **25**: 40. 1935.

**Chronica Botanica, the year-book of plant science has been designed to bring together the research programmes of all laboratories, experimental stations, herbaria, botanic gardens, etc., to give a résumé every April of the professional and personal news of the past year and to promote co-operation between workers in the various branches of plant science in every possible way.**

**Chronica Botanica** deals not only with general botany, taxonomy, ecology, etc., but also with agriculture, agronomy, forestry, horticulture, phytopathology, genetics, plant breeding, microbiology, soil science, agricultural bacteriology and chemistry, plant-biochemistry and pharmacognosy.

**Chronica Botanica** contains a complete, annotated and up-to-date list of all laboratories, expt. stations, herbaria, botanical gardens and societies connected with every branch of plant science. Special sections are devoted to the Internat. Bot. Congress and to other internat. congresses, committees and societies. It also includes an almanac of events, past, present and future, a section for correspondence, notes on new periodicals, lists of new and changed addresses and indices of names of plants, persons and societies.

**Chronica Botanica** *sends questionnaires every December to the Directors of all botanical laboratories, expt. stations, herbaria and gardens and to the Secretaries of all botanical societies. Answers must reach the Editor before the end of January; it is impossible to use information received after that date.*

> CHRONICA BOTANICA is published every April as a single volume of about 400 pages, with numerous illustrations, bound in cloth. Annual subscription hfl. 15.-, postage extra. For prospectus, sample pages and further information, apply to the Editorial and Publishing Office, P. O. Box 8, Leiden, Holland.

**This year-book is the first and only one of its kind : it contains a vast amount of hitherto unavailable information. It answers hundreds of questions which you have previously had to leave unanswered, though they were of the greatest importance for your work. An annual subscription will well repay you - you cannot afford to be without the latest issue of the "Chronica".**